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14. ABSTRACT
How leukemia stem cells gained resistance to radiation and chemotheraphy is poorly defined, yet critically determines how leukemia cells tolerate conventional leukemia therapy. Normal hematopoietic stem cells and leukemia initiating cells are known to share many functional properties. Therefore, they are supposed to utilize many common mechanistic pathways for their survival and migration. Using genetically engineered mice we demonstrated the functional roles of P2Y14 in preserving regenerative capacity by constraining senescence induction and molecular events governing it. Since P2Y14 is highly expressed in differentiation-resistant leukemia cells, P2Y14 expression in leukemia cells may also function in modulating the resistance to conventional cancer treatment. We believe these data define for the first time in mammals the identity and impact of a receptor modulating stem/progenitor tolerance of stress. By providing a mechanistic insight for the roles of P2Y14 in the stress-induced injury, our preliminary results are expected to provide the foundation for an effective treatment to destroy therapy resistant leukemia cells. In addition, we identified a nucleotide sugar, UDP-Glc, as a novel mobilizer of long-term repopulating HSPCs. This finding may also have important clinical implications in designing new mobilization strategies to improve the efficiency and outcome of autologous and allogeneic peripheral blood stem cell (PBSC) transplantation.

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Introduction

Although conventional therapy temporarily lessens the burden of the disease, a lingering subpopulation of drug- and radiation-resistant leukemia may regenerate. This small subpopulation of drug- and radiation-resistant leukemia is an immediate concern for leukemia patients as this subtype remains the actual cause of morbidity and mortality.

Nucleotides, once recognized as mere sources of energy, are now emerged as key extracellular messengers that regulate diverse aspects of homeostasis in various physiological and pathophysiological conditions (Volonte et al., 2006). Extracellular nucleotides exert their actions through interaction with their cognate receptors, purinergic receptors. Purinergic receptors are classified into P1 and P2 receptors, based on their ligand binding and function (White and Burnstock, 2006). The role of P2 receptors as regulators of hematopoiesis has become more evident in recent years (Di Virgilio et al., 2001b; Sak et al., 2003). A wide variety of P2 receptors are expressed in blood and inflammatory cells, and their physiological significance has been demonstrated (Di Virgilio et al., 2001a).

Recent results defined molecular signatures predicting the drug resistance of leukemia cells (Tagliafico et al., 2006). $P2Y_{14}$ expression has been shown to be highly upregulated in differentiation-resistant acute myeloid leukemia (AML) cases in 28 freshly isolated AML blast populations, making the $P2Y_{14}$ gene a prime suspect of incurable leukemia. $P2Y_{14}$ is also listed as a target gene of the *Wnt3A*, whose aberrant regulation is closely associated with hematological malignancies and several types of other cancers (Nygren et al., 2007). In this report, when leukemia cells are treated with Wnt3A, $P2Y_{14}$ was the gene most strongly upregulated. More recently, a comprehensive mutational analysis of human cancer identified $P2Y_{14}$ as one of the candidate cancer genes that is mutated at a significant frequency in a large fraction of colorectal cancers (Sjoblom et al., 2006).

It is believed that a similar set of genes controls both normal and cancer stem cells. Therefore, if the genes expressed by normal stem cells are found to be mutated or used differently in cancer cells, it is very likely that those genes play a role in the development of cancer stem cells. Our preliminary findings demonstrate a novel role for $P2Y_{14}$ in the response to radiation and chemo reagents, serving as a modifier of cell senescence and cell death, thereby enabling preservation of hematopoietic stem/progenitor cell (HSPC) function. Considering the similarity between normal and leukemic stem cells, our preliminary results lead to the likelihood

that $P2Y_{14}$ is closely associated not only with maintenance of the normal HSC but also with the drug-resistant leukemia cells.

Bone marrow transplantation (BMT) is a potentially everlasting curative therapy for hematological diseases such as leukemia, lymphoma and various types of immunologic disorders. In the past years, bone marrow cells have been replaced by mobilized peripheral blood stem cells (PBSCs), because PBSCs engraft better than bone marrow-derived HSPCs and allow faster recovery of the white blood cell count. With faster engraftment and reduced risk of posttransplant infection, mobilized cells became a major source of HSPCs for autologous and allogeneic transplantations. Our preliminary findings demonstrate that UDP-Glucose, a putative ligand of $P2Y_{14}$ receptor, has a previously unknown function in mediating HSPC mobilization.

Taken together, we propose that the $P2Y_{14}$ receptor is an important local regulatory molecule that leads to both normal and leukemic stem cell migration and quiescence and the manipulation of $P2Y_{14}$/UDP-Glucose signaling axis may modulate the susceptibility of normal and leukemic stem cells to radiation and chemo reagents.

Body

Specific Aim 1: To investigate how $P2Y_{14}$ signaling axis regulates the quiescence of LSC

It is hypothesized that leukemic stem cells arise either from normal stem cells or from progenitor cells. We have investigated whether $P2Y_{14}$ mediates the resistance of stem progenitor cells to irradiation (IR) or chemo reagents using *P2ry14* knock-out (KO) mouse model system. In our previous annual reports, we demonstrated that *P2ry14* deficiency confers hypersusceptibility to IR and chemotherapy drug, such as 5-flurouracil (5-FU).

Since different doses of IR can produce different biological impacts, we further examined the effects of low- (3Gy), medium- (6Gy, near LD50) and high (8Gy) doses of radiation on the IR-induced cell injury. 3 Gy TBI did not cause a statistically significant difference in IR-induced cell death between WT and KO LSK cells (Figure 1). At doses of greater than 8 Gy, only a very small number of HSPCs were alive (data not shown), making further analysis difficult. At 5-6 Gy TBI a distinct effect on $P2Y_{14}$ deficient LSKs compared with WT counterparts was noted (Figure. 1). Others have documented that TBI at those doses rarely resulted in animal death, but

induced both acute and long-term hematopoietic dysfunction (Wang et al., 2006). Therefore, we used 6 Gy TBI to investigate the role of $P2Y_{14}$ receptor.

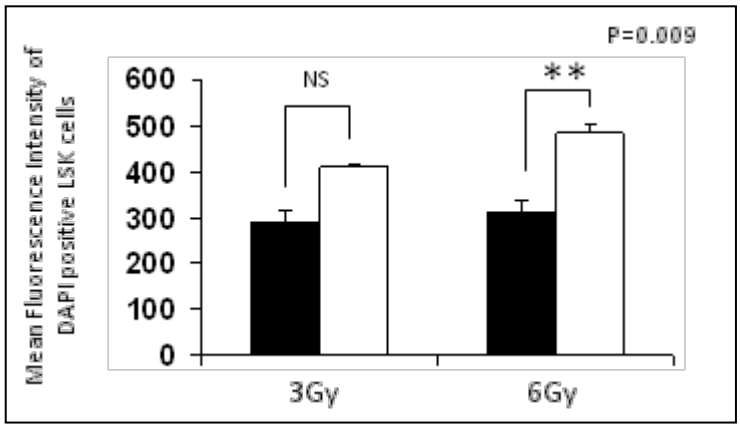

Figure 1. Mice of the indicated genotypes were exposed to 3 Gy and 6 Gy of TBI. Cell death was measured by quantification of DAPI positive cells within LSK cells. The data are representative of three independent experiments. At doses of greater than 8 Gy, only a very small number of LSK cells were alive (data not shown), making it difficult to obtain enough cells for the further analysis. Therefore, TBI doses greater than 8 Gy were not selected for the further study.

We previously reported that the preferential diminution of $P2Y_{14}$ KO HSPCs under radiation stress was due to the increased susceptibility of $P2Y_{14}$ KO HSPCs to IR-induced cell death and senescence.

In addition, we reported that the significantly higher levels of mitochondrial superoxide were detected in KO HSPCs both *in vivo* and *in vitro* upon irradiation. The increased ROS level in $P2Y_{14}$ KO LSK cells coincided with a low mitochondrial membrane potential ($\Delta\psi m$). N-acetyl-cysteine (NAC), the ROS scavenger, treatment reduced levels of superoxide in LSK cells and alleviated the IR-induced cell death and senescence in $P2Y_{14}$ deficient LSK cells. This was accompanied with restoration of BM cellularity and LSK cell number.

When cellular senescence is induced, there are typical expression patterns of the cell-cycle regulators (Wei et al., 2003) including the cyclin dependent kinase inhibitors such as $p16^{INK4a}$ (Alcorta et al., 1996). We did not detect differences in expression levels of $p16^{INK4a}$ transcripts in freshly prepared $P2Y_{14}$ KO and WT LSK cells (data not shown). When KO cells were transplanted into primary recipients, $p16^{INK4a}$ expression began to increase in $P2Y_{14}$ KO LSK cells (a moderate 1.9 fold increase compared with that in WT LSK) (p=0.003) (Figure 2A,

upper panel). When these primary recipient animals were subjected to subsequent radiation, $P2Y_{14}$ KO LSK cells displayed a marked increase in *p16*^{INK4a} expression (6-8 fold, P=0.006) (Figure 2A, lower panel). The observed changes in *p16*^{INK4a} transcript were paralleled by changes in p16^{Ink4a} protein in LSK and SLAM LSK cells (Figure 2B).

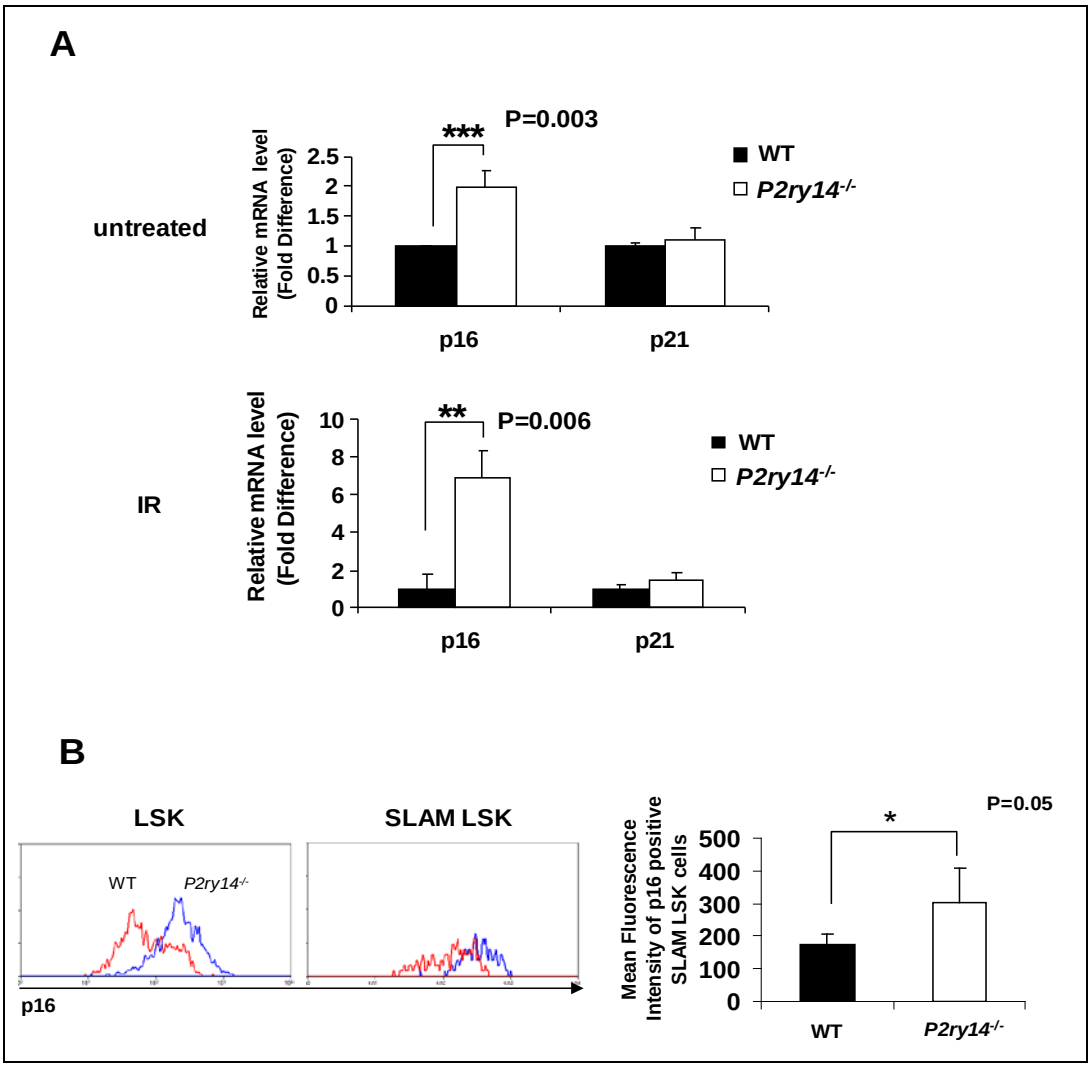

Figure 2: (A) WT and *P2ry14*$^{-/-}$ BM cells were transplanted into recipient mice. Eight months after transplantation, recipient mice were either left untreated (n=4) or irradiated (n=4) with TBI (6 Gy). LSK cells derived from WT (CD45.1+ LSK) or *P2ry14*$^{-/-}$ (CD45.2+ LSK) donors in the recipients were sorted and then subjected to quantitative PCR analysis, respectively. The expression level in WT cells was arbitrarily set to 1. The fold change in expression of each gene was calculated using the ΔΔCt method. The expression was normalized to GAPDH. (B) Mice of the indicated genotypes were exposed to TBI (6Gy). After 4 weeks of TBI, LSK and SLAM LSK cells were gated and analyzed for the expression of p16^{Ink4a} by flow cytometry analysis. Mice were analyzed individually, and values shown are the mean ± s.d. (n >3 mice/group, right panel). Representative flow cytometric analysis of p16^{Ink4a} in gated LSK and SLAM LSK cells is shown (left panel).

HSPCs isolated from UDP-Glucose (UDP-Glc), a putative ligand for $P2Y_{14}$ receptor, treated mice contained a significantly higher proportion of cells in G0 and lower proportion in the G1, S, and G2/M phases compared to counterparts from control mice (Figure 3), suggesting that $P2Y_{14}$ signaling axis is associated with the cell cycle quiescence of HSPCs. This result supports our hypothesis that $P2Y_{14}$ axis plays a role in regulating the cell cycle quiescence of stem progenitor cells.

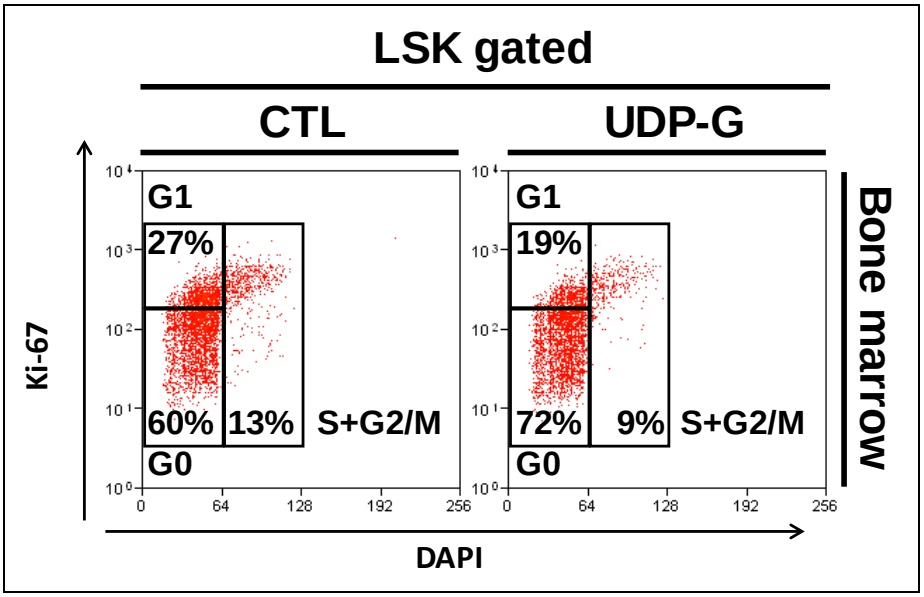

Figure 3: The effect of UDP-Glc on cell cycle status of HSPCs was evaluated using LSK cells from control (vehicle-injected) or UDP-Glc-injected mice. Mice were injected once daily with UDP-Glc (200mg/kg body weight) or PBS for 6 days. The bone marrow samples were pooled from each group (n>4) and stained for Ki67 and DAPI. LSK cells were pregated and further analyzed for their cell cycle status.

Taken together, our preliminary results strongly suggest that the presence of $P2Y_{14}$ in stem/progenitor cells may determine radio and chemo-resistance of cells. Based on these results, we will continue to investigate whether $P2Y_{14}$ axis is associated with therapy resistant leukemia phenotypes using the chimeric mice established in Specific Aim 2. This was not yet determined due to the low percentages of xenografted human leukemia cells in the recipient mice. We are currently focusing our efforts on improving homing and engraftment of transplanted human leukemia cell.

Specific Aim 2: To investigate functional correlation between P2Y14 signaling and therapy-resistant leukemia

In the previous reports, we determined whether P2Y14 KO HSPCs display differential activation of p38 MAPK under genotoxic stress. Whereas there was no detectable difference in p38 MAPK activity between WT and KO HSPCs under homeostatic conditions, p38MAPK activity was notably higher in $P2Y_{14}$ deficient LSK and SLAM LSK cells following radiation (6 Gy, TBI). NAC treatment almost completely abolished the p38 M APK activation. While we were unable to detect JNK activation due to the lack of antibodies that work for flow cytometry, the JNK inhibitor also significantly reduced levels of IR-induced cellular senescence in KO HSPCs (Figure 4A). These results indicate that JNK activation may also play a significant role in modulating the susceptibility of $P2Y_{14}$ deficient LSK cells to IR. Interestingly, while the JNK inhibitor diminished IR-induced senescence (Figure 4A), it did not have a substantial effect in restoring colony-forming activity of irradiated $P2Y_{14}$ deficient LSK cells (Figure 4B). These data raise the intriguing possibility that while the activation of p38 MAPK induces cell death and senescence, JNK activation is associated only with senescence in $P2Y_{14}$ deficient LSK cells.

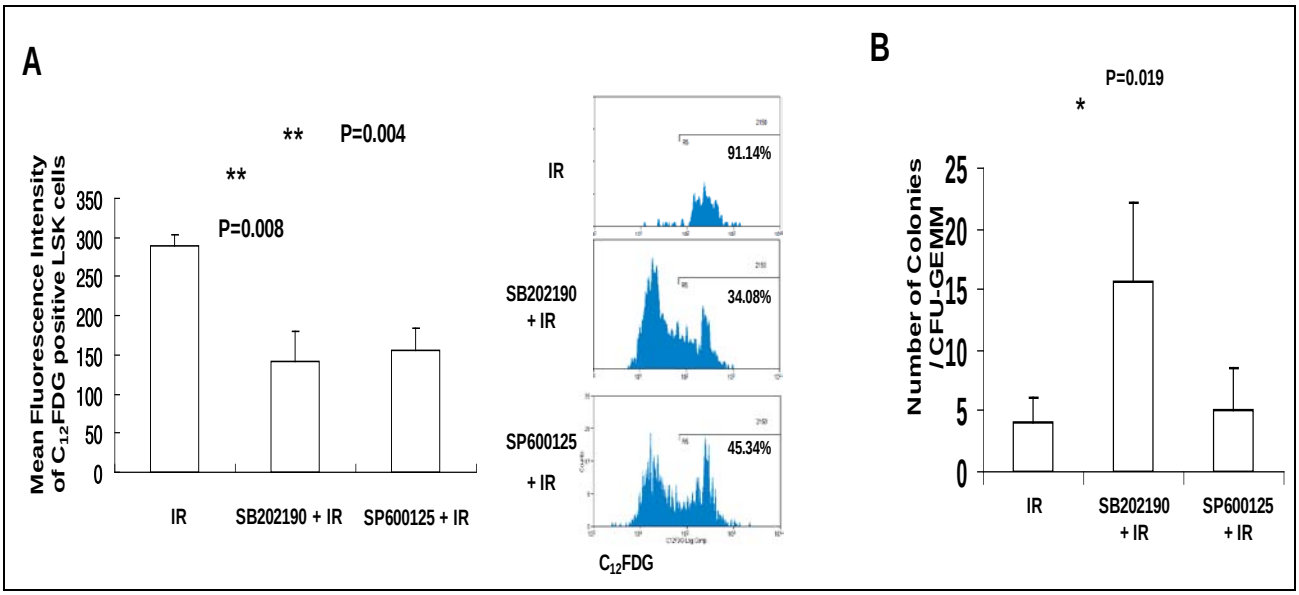

Figure 4: WT and *P2ry14*$^{-/-}$ mice were exposed to TBI (6Gy). p38 MAPK inhibitor (SB203580, 2 mg/Kg) or JNK inhibitor (SP-600125) (10 mg/Kg) was administered 30 min before and immediately after TBI. (A) LSK cells were analyzed at 4 weeks posttransplantation for C_{12} FDG expression. (B) Colonogenic capacity of the treated cells was analyzed by colony formation unit assay (CFU-GEMM). Experiments were repeated at least twice with similar results.

Taken together, our data demonstate that $P2Y_{14}$ deficiency confers increased susceptibility to genotoxic stress-induced cell death and senescence in HSPC compartments via the excessive activation of p38 MAPK and JNK triggered by IR.

Aberrant activation of p38 MAPK and JNK pathways are potential causes of hematologic malignancies. Therefore, we investigated whether P2Y14/UDP-Glc axis can trigger p38 MAPK and/or JNK signaling pathway in leukemia cells as shown in normal HSPCs. As shown previously, UDP-Glc triggered the activation of both p38 MAPK and JNK pathway in KG-1 leukemia cells starting as early as 5 min after UDP-Glc treatment. For JNK proteins, the activation peaked at 30 min post-treatment and decreased thereafter. Interestingly, p38 MAPK activity reached a peak after 5 min and then sustained its activity over prolonged time. We are currently assessing the impact of these pathways on cell death and senescence in leukemia cells.

To investigate whether UDP-Glc induces the activation of p38 MAPK and JNK in primary human leukemia cells, we transplanted human acute leukemia samples (AML; M0 or M1 stage) into NOD-*scid IL2R*γ^{null} mice (n>30). We have obtained bone marrow samples from 41 unidentified leukemia patients. Among these samples, five human acute leukemia samples (AML) (M0 or M1) were chosen based on FAB classification, cytogenetic abnormalities, CD34 and CD14 expression (Table 1). Among AML cells transplanted, three AML samples showed detectable human leukemia cells, albeit at a low level. We performed secondary transplantation to assess whether transplanted cells contain leukemia-initiating cells. While we were carrying out the serial transplantation experiments, Rotavirus (Epizootic diarrhea of infant mice) was identified in our animal facility. Since our recipient animals (NOD-*scid IL2R*γ^{null}) are immunodeficient, all of the recipient animals had to be euthanized. We will resume transplantation experiments after the remediation burn out period (3-4 months). As proposed, we will continue to test whether the activation of $P2Y_{14}$/UDP-Glc signaling confers drug resistance (ex: against Ara-C treatment) to xenotransplanted human leukemia cells.

Patient	*Age*	*FAB*	*Cytogenetic abnormalities*	*Blast*	*CD34+*	*CD14+*
104	66	M0/M1	Negative PML-RARA (unlike M2)	92%	35%	0%
133	60-64	M0	ETV6 (TEL) rearrangement t(12;22) (Q13;Q11.2) t(6;15), del(17)	63%	N/A	6%
069	30	M0	11q23 t(11;19)(q23;p13.3), +19	87%	0%	0%
084	69	M0	2+, 3-, 5-, -7, +8, -14, -15 etc. Complex karyotypes	30%	63%	N/A
170	60-64	M0/M1	-4, t(7:11) -6, MLL(11q23)x3, MLLx4~6 etc.	90%	92%	15%

Table 1: Morphologic, immunologic, and cytogenetic classification of AML samples

Specific Aim 3: Examine whether the activation of $P2Y_{14}$/UDP-Glucose signaling axis mobilizes leukemic stem cells from recipient's bone marrow.

Mobilized HSPCs are a major source of peripheral stem cell transplantation (PSCT) for leukemia patients. Our results demonstrate that a nucleotide sugar, UDP-Glc, which is known to release into extracellular fluids in response to various stressors, has a previously unknown function in mediating HSPC mobilization. With faster engraftment and reduced risk of posttransplant infection, mobilized HSPCs are now more commonly used as stem cell sources.

In our previous two progress reports we showed the ability of UDP-Glc to mobilize CFU-Cs and LSKs into the blood circulation. There was a noticeable increase in CFU-Cs after 6 daily single UDP-Glc injections.

G-CSF is the most commonly used cytokine for mobilization of HSPCs in the clinic. We thus determined the mobilizing capability of UDP-Glc in comparison with G-CSF. Importantly, the frequency of LSKs in the blood of mice treated with UDP-Glc was similar to that obtained with G-CSF treatment as shown in previous reports. UDP-Glc mobilized cells not only displayed the ability to rescue lethally irradiated recipients but also were far superior to control blood cells in a competitive repopulation assay (Figure 5A). The maintenance of stem cell pool and generation of functional mature blood cells depend on c lose interaction with specialized microenvironments or niches in bone marrow (Purton and Scadden, 2006). Therefore, the

engraftment of HSPCs to bone marrow more accurately represents clinical outcome in clinical protocols. We thus assessed whether donor-derived HSPCs are sustainable in the bone marrow of recipient animals for an extended period after transplantation. We analyzed the bone marrow of recipient animals at 18 weeks after transplantation. A significantly higher portion of LSK and SLAM LSK cells in recipient bone marrow were derived from UDP-Glc-treated mice at 18 weeks after transplantation (Figure 5B and 5C), indicating that UDP-Glc- mobilized cells achieved higher levels of long-term engraftment than G-CSF-mobilized cells.

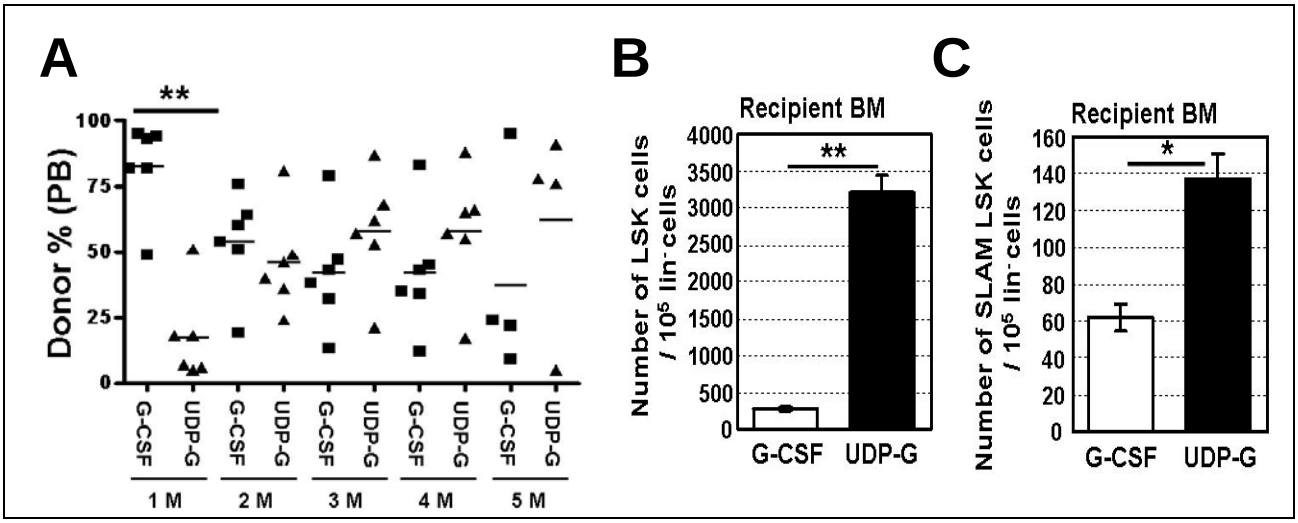

Figure 5: (A) Mice were injected s.c. once daily with UDP-Glc (200 mg/kg, 6 days) or G-CSF (300 µg/kg, 4 da ys). The competitive repopulation assay was performed. UDP-Glc-mobilized blood cells ($2X10^6$) were mixed with an equal number of G-CSF-mobilized blood cells, and then transplanted into conditioned (10 Gy TBI) recipient animals. The contribution of donor cells was measured at the indicated times after transplantation. (B-C) Eighteen weeks after transplantation, bone marrow cells were obtained from recipient animals and analyzed by flow cytometry for LSK (3B) and SLAM LSK (3C) cells after gating on CD45.1+ (G-CSF injected) and CD45.2+ (UDP-Glc injected) cells. Data are expressed as number of LSK (upper panel) and SLAM LSK (lower panel) cells per 10^5 lineage-negative cells. The data shown are the mean ± SD. *$p < 0.05$ and ** $p < 0.01$

The preferential engraftment of long-term repopulating cells with UDP-Glc-mobilized cells may indicate the possibility that UDP-Glc mobilizes a more primitive subset of HSPCs such as SLAM LSK cells than G-CSF. UDP-Glc promoted LSK cell mobilization into the peripheral blood, with efficacy similar to that of G-CSF (0.048% vs. 0.058%) (Figure 6). However, UDP-Glc-mobilized LSK cells contained a significantly higher proportion of SLAM LSK cells compared to that of G-CSF-mobilized cells (0.0065% vs. 0.0014 %) (Figure 6).

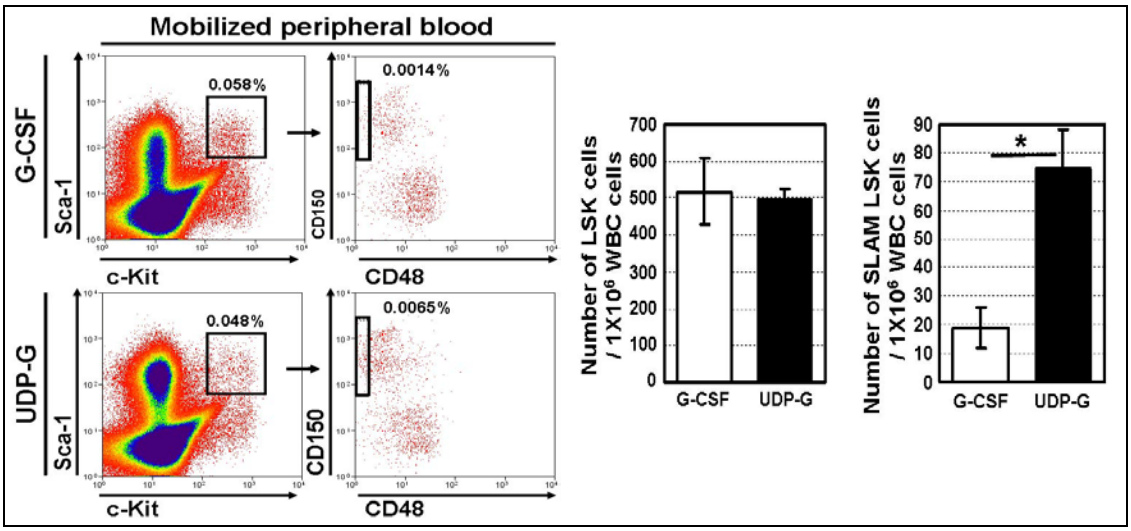

Figure 6: Mice were injected s.c. once daily with UDP-Glc (200 mg/kg, 6 days) or G-CSF (300 µg/kg, 4 days). Peripheral blood cells were analyzed by flow cytometry for LSK and SLAM LSK cells. Left: Representative flow cytometry plots showing the frequency of LSK and SLAM LSK cells in mobilized peripheral blood. Numbers indicate the percentage of gated cells within the total number. Right: Data are expressed as number of LSK and SLAM LSK cells per 10^6 peripheral blood mononuclear cells. Data shown are pooled data from two independent experiments with four to five mice per group. The data shown are the mean ± SD. *$p < 0.05$

Serial transplantation represents the gold standard for assessing the long-term repopulation abilities. In order to further compare the long-term repopulation abilities of UDP-Glc- and G-CSF-mobilized HSPCs, we performed serial transplantation experiments under competitive settings. Primary recipients were transplanted with UDP-Glc (CD45.2)- and G-CSF (CD45.1)-mobilized peripheral blood cells as shown in Figure 5. At 2-3 months post-transplant, bone marrow cells from primary recipients were sorted based on their expression of CD45. A mixture of equal numbers of CD45.1 (derived from G-CSF mobilization) and CD45.2 (derived from UDP-Glc mobilization) bone marrow cells were then transplanted into lethally irradiated secondary recipients. While G-CSF-mobilized peripheral blood cells have superior short-term repopulating ability in primary recipient animals (Figure 5), they were completely out-competed by the cells derived from UDP-Glc mobilization in the secondary recipients over the whole post-transplantation period (Figure 7). These data reinforce the hypothesis that UDP-Glc-mobilized HSPCs have enhanced self-renewal capacity when compared to G-CSF-mobilized HSPCs. Furthermore, these data support the notion that UDP-Glc mobilizes a functionally distinct subset of HSPCs.

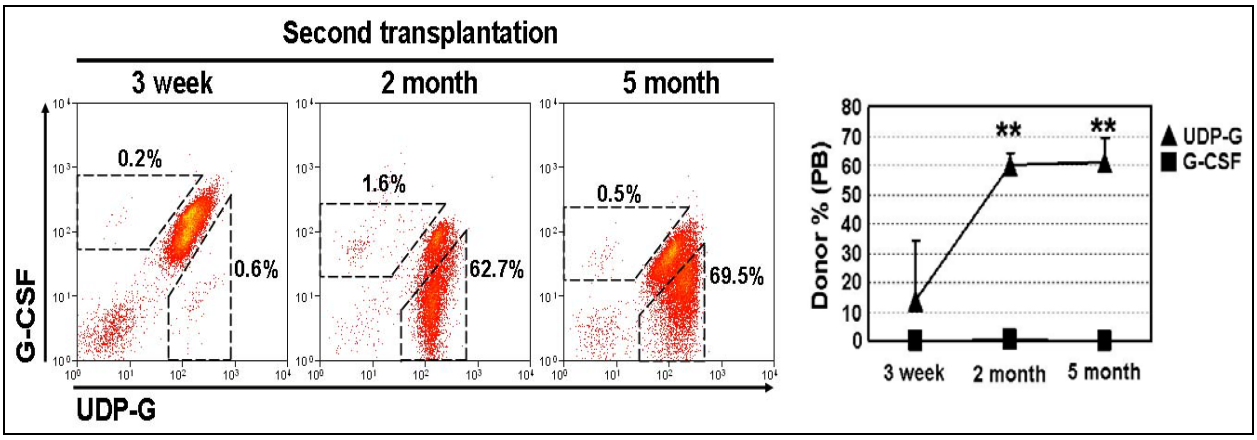

Figure 7: Primary recipients (CD45 1.2) were transplanted as described in Figure 5. Two to three months after transplantation, bone marrow cells from primary recipients were sorted based on their expression of CD45. A mixture of equal numbers of bone marrow cells-derived from UDP-Glc-(CD45.2) and G-CSF (CD45.1)–mobilization were transplanted into secondary recipients (CD45 1.2). Donor cell chimerism in the recipient mice was analyzed at the indicated times post-transplant.

There is a keen interest in improving the mobilizing effects of G-CSF (Broxmeyer et al., 2005). Therefore, we investigated possible functional synergies between UDP-Glc and G-CSF. The mobilizing effect of UDP-Glc peaked 2-4 hours after the sixth daily consecutive injection. G-CSF requires four consecutive daily injections (Broxmeyer et al., 2005). Based on these results, the administration schedule of the compounds was designed to synchronize the maximal effect of each treatment as shown in Figure 8A. In the setting of competitive repopulation assay, UDP-Glc/G-CSF-mobilized cells were dominant over G-CSF-mobilized cells throughout the whole post-transplantation period (Figure 8B). Although UDP-Glc alone was not as efficient as G-CSF in mobilizing *in vitro* colony forming HPCs as shown in our previous reports, a combination of UDP-Glc and G-CSF markedly enhanced short-term repopulating activity compared with G-CSF alone, and this competitive advantage was continued over at least 5 months after transplantation (Figure 8B). Accordingly, a significantly higher portion of LSK and SLAM LSK cells in recipient bone marrow were derived from UDP-Glc/G-CSF-treated mice (Figure 8C and 8D).

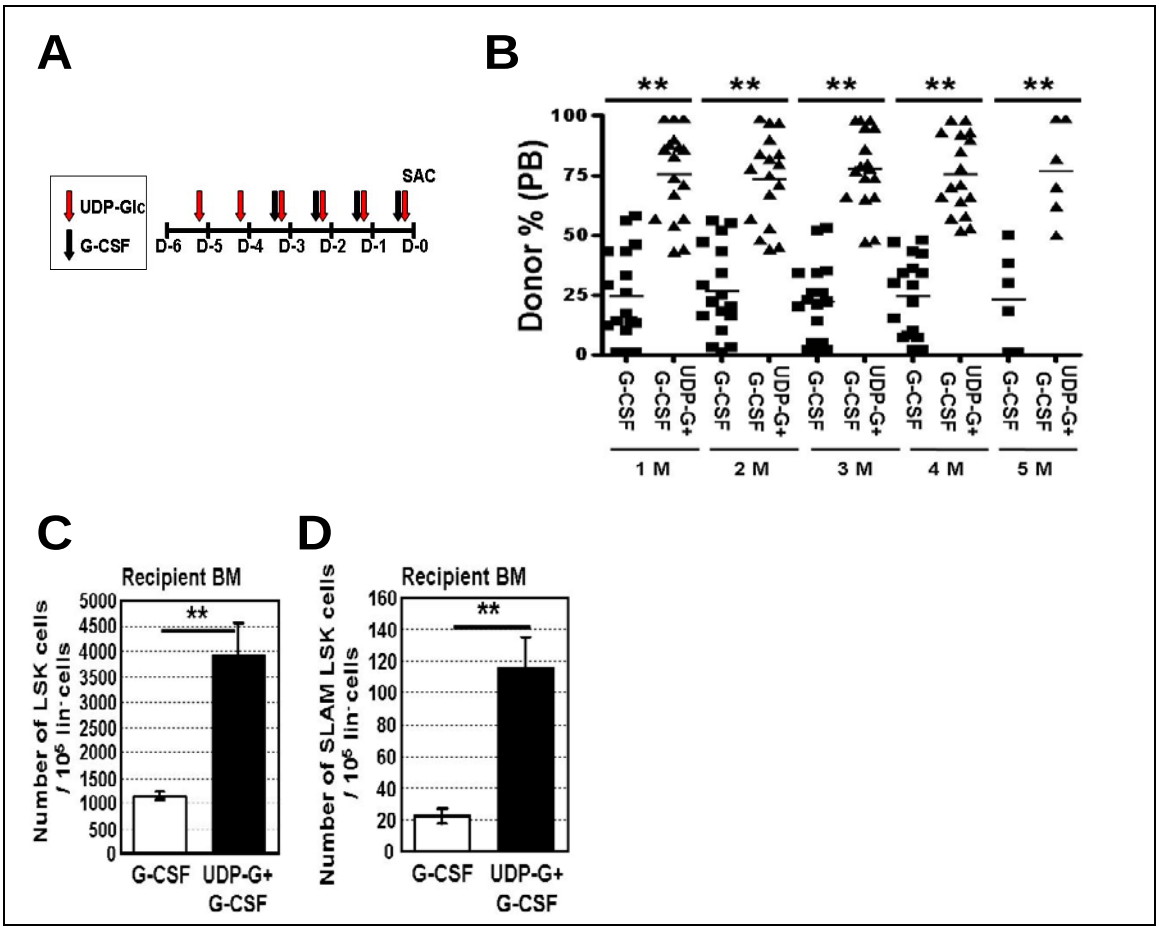

Figure 8: (A) Schema of combinatorial administration schedule: G-CSF was injected daily for 4 consecutive days. UDP-Glc was injected daily for 6 consecutive days. Mice were sacrificed (day 0. SAC) and blood cells were further analyzed for hematopoietic stem progenitor cell activity. (B) Mice were treated as described in (A). Peripheral blood cells were collected from G-CSF- and UDP-Glc/G-CSF-injected mice and transplanted in equal numbers into lethally irradiated recipient animals. The contribution of donor cells in the peripheral blood of the recipient animals was assessed at the indicated times as described in Figure 5. ** $p < 0.01$. (C-D) Eighteen weeks after transplantation, bone marrow cells were obtained from recipient animals (n=5) and analyzed by flow cytometry for LSK (C) and SLAM LSK (D) cells. Data are expressed as number of LSK and SLAM LSK cells per 10^5 lineage-negative cells. The data shown are the mean ± SD. ** $p < 0.01$

Mobilized HSPCs could regenerate a complete hematopoietic system for cancer patients with hematolymphoid malignancies or solid tumors. Yet more than 20 percent of patients fail to mobilize sufficient stem cells for transplantation (Schmitz et al., 1996). These include patients who were previously treated with intensive radiation and chemotherapy; those who have genetic disorders such as Fanconi's anemia; and those who are over 60 years of age (Broxmeyer et al., 2005; Cottler-Fox et al., 2003).

UDP-Glc is a natural product, so that it may mitigate many of the side effects which are often associated with other synthetic mobilizers. Indeed, none of the UDP-Glc-treated animals showed signs of side effects and appeared normal during the course of the study. The small size of UDP-Glc also offers other tangible advantages over other protein-based mobilizers, including easy access to intracellular targets and low cost and ease of production as well as oral bioavailability.

Key Research Accomplishments

1. $P2Y_{14}$ functions in bone marrow to preserve hematopoietic stem/progenitor cells from premature senescence and cell death induced by genotoxic stress.
2. We identified potential mechanisms by which $P2Y_{14}$ signaling axis mediates radiation and chemo reagent resistance.
3. We demonstrate that UDP-Glucose has a previously unknown function in mediating HSPC mobilization.

Reportable Outcomes

Cho JS, Shen H, Hui Y, Cheng T, Lee SB, Lee BC. 2011. Ewing's Sarcoma Gene EWS regulates Hematopoietic Stem Cell Senescence. *Blood,* 117:1156-66.

Kook SH, Cho JS, Lee BC. A Nucleotide Sugar, UDP-Glucose, is a Novel Mobilizer of Long-Term Repopulating Primitive Hematopoietic Cells. *Journal of Clinical Investigation,* (In Revision)

Conclusion

How leukemia stem cells gained resistance to radiation and chemotheraphy is poorly defined, yet critically determines how leukemia cells tolerate conventional leukemia therapy. Normal hematopoietic stem cells and leukemia initiating cells are known to share many functional properties. Therefore, they are supposed to utilize many common mechanistic pathways for their survival and migration. Using genetically engineered mice we demonstrated the functional roles of $P2Y_{14}$ in preserving regenerative capacity by constraining senescence induction and molecular events governing it. Since $P2Y_{14}$ is highly expressed in differentiation-resistant leukemia cells, $P2Y_{14}$ expression in leukemia cells may also function in modulating the resistance to

conventional cancer treatment. We believe these data define for the first time in mammals the identity and impact of a receptor modulating stem/progenitor tolerance of stress.

By providing a mechanistic insight for the roles of $P2Y_{14}$ in the stress-induced injury, our preliminary results are expected to provide the foundation for an effective treatment to destroy therapy resistant leukemia cells.

In addition, we identified a nucleotide sugar, UDP-Glc, as a novel mobilizer of long-term repopulating HSPCs. This finding may also have important clinical implications in designing new mobilization strategies to improve the efficiency and outcome of autologous and allogeneic peripheral blood stem cell (PBSC) transplantation.

References

Alcorta, D.A., Xiong, Y., Phelps, D., Hannon, G., Beach, D., and Barrett, J.C. (1996). Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. Proc Natl Acad Sci U S A *93*, 13742-13747.

Broxmeyer, H.E., Orschell, C.M., Clapp, D.W., Hangoc, G., Cooper, S., Plett, P.A., Liles, W.C., Li, X., Graham-Evans, B., Campbell, T.B., *et al.* (2005). Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. J Exp Med *201*, 1307-1318.

Cottler-Fox, M.H., Lapidot, T., Petit, I., Kollet, O., DiPersio, J.F., Link, D., and Devine, S. (2003). Stem cell mobilization. Hematology Am Soc Hematol Educ Program, 419-437.

Di Virgilio, F., Borea, P.A., and Illes, P. (2001a). P2 receptors meet the immune system. Trends Pharmacol Sci *22*, 5-7.

Di Virgilio, F., Chiozzi, P., Ferrari, D., Falzoni, S., Sanz, J.M., Morelli, A., Torboli, M., Bolognesi, G., and Baricordi, O.R. (2001b). Nucleotide receptors: an emerging family of regulatory molecules in blood cells. Blood *97*, 587-600.

Nygren, M.K., Dosen, G., Hystad, M.E., Stubberud, H., Funderud, S., and Rian, E. (2007). Wnt3A activates canonical Wnt signalling in acute lymphoblastic leukaemia (ALL) cells and inhibits the proliferation of B-ALL cell lines. Br J Haematol *136*, 400-413.

Purton, L.E., and Scadden, D.T. (2006). Osteoclasts eat stem cells out of house and home. Nat Med *12*, 610-611.

Sak, K., Boeynaems, J.M., and Everaus, H. (2003). Involvement of P2Y receptors in the differentiation of haematopoietic cells. J Leukoc Biol *73*, 442-447.

Schmitz, N., Linch, D.C., Dreger, P., Goldstone, A.H., Boogaerts, M.A., Ferrant, A., Demuynck, H.M., Link, H., Zander, A., and Barge, A. (1996). Randomised trial of filgrastim-mobilised peripheral blood progenitor cell transplantation versus autologous bone-marrow transplantation in lymphoma patients. Lancet *347*, 353-357.

Sjoblom, T., Jones, S., Wood, L.D., Parsons, D.W., Lin, J., Barber, T.D., Mandelker, D., Leary, R.J., Ptak, J., Silliman, N., *et al.* (2006). The consensus coding sequences of human breast and colorectal cancers. Science *314*, 268-274.

Tagliafico, E., Tenedini, E., Manfredini, R., Grande, A., Ferrari, F., Roncaglia, E., Bicciato, S., Zini, R., Salati, S., Bianchi, E., *et al.* (2006). Identification of a molecular signature predictive of sensitivity to differentiation induction in acute myeloid leukemia. Leukemia *20*, 1751-1758.
Volonte, C., Amadio, S., D'Ambrosi, N., Colpi, M., and Burnstock, G. (2006). P2 receptor web: complexity and fine-tuning. Pharmacol Ther *112*, 264-280.
Wang, Y., Schulte, B.A., LaRue, A.C., Ogawa, M., and Zhou, D. (2006). Total body irradiation selectively induces murine hematopoietic stem cell senescence. Blood *107*, 358-366.
Wei, W., Herbig, U., Wei, S., Dutriaux, A., and Sedivy, J.M. (2003). Loss of retinoblastoma but not p16 function allows bypass of replicative senescence in human fibroblasts. EMBO Rep *4*, 1061-1066.
White, N., and Burnstock, G. (2006). P2 receptors and cancer. Trends Pharmacol Sci *27*, 211-217.

Appendices

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